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Protogynous Sex Change in the Intertidal Isopod *Gnorimosphaeroma oregonense* (Crustacea: Isopoda)

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Abstract. In Crustacea, the dominant pattern of sequential hermaphroditism is protandry (sex change from male to female). Here we provide the first evidence from external morphology and population structure that *Gnorimosphaeroma oregonense*, an abundant, sexually dimorphic intertidal isopod, undergoes protogynous (female to male) sex change. In the field, 31% of females had rudimentary penes, suggesting sex change, and laboratory growth experiments confirmed that females produced one brood of juveniles, then passed through a variable number of molts as immature males before becoming sexually mature males. Contrary to reports for other protogynous Crustacea, this study suggests that in *G. oregonense* sex change is not socially mediated, although it may be facultative, because a large percentage of laboratory-reared juvenile isopods developed directly into males. Potential adaptive explanations for protogyny are discussed in relation to protandry—the more common strategy in Crustacea.

Introduction

Although sex change occurs in only a small percentage of species, protandry (change from male to female) and protogyny (change from female to male) have been documented in a diverse assemblage of plant and animal taxa (Ghiselin, 1969; Policansky, 1982). However, either protandry or protogyny tends to predominate among closely related species. In the Crustacea, sex change is predominantly protandrous: 82% of the 60 sequentially hermaphroditic species listed in Table I undergo sex change from male to female. Protandry has been reported in the classes Cirripedia; Copepoda; and within the Malacostraca in nine families of Decapoda, two families of Amphipoda, and four families of Isopoda (Table I). Protogyny has been reported in five tanaidacean and four isopod species (Table I); thus both protandry and protogyny have likely developed independently several times in the Crustacea. Protandry can be advantageous when female fecundity increases with age or size but male mating success remains independent of size (Warner, 1975; Warner, 1988). Conversely, protogyny may arise when small males are prevented from mating with females by larger males, making it advantageous to become male only when a competitive larger size is reached (Warner, 1988).

The Isopoda is the only crustacean order in which both protandrous and protogynous species occur (Table I), giving the opportunity to compare closely related species exhibiting both forms of sequential hermaphroditism. In marine isopods, protogynous sex change appears, based on a limited number of species examined (Table I), to be restricted to free-living forms (Legrand and Juchalt, 1963; Burbanck and Burbanck, 1974; Buss and Iverson, 1981); whereas protandry occurs primarily within the parasitic suborders Epicarida and Flabellifera (Family Cymothoidae; Brusca, 1981).

In this study we examine sex change in the free-living marine isopod *Gnorimosphaeroma oregonense* Dana 1852, a common inhabitant of protected rocky intertidal shores ranging from Alaska to northern California (Hoestlandt, 1973). Although this species has been the subject of numerous physiological (Riegel, 1959a,b;

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Table 1

Summary of the occurrence of protandrous and protogynous sex change within the Crustacea

Taxa[1]	Reference	Type of sex change: Protandry	Type of sex change: Protogyny
Class Malacostraca			
Subclass Eucarida			
O. Decapoda			
S.O. Pleocyemata			
I.O. Caridea			
Alpheidae			
Athanas kominatoenis	Suzuki, 1970	X	
Atyidae			
Paratya curvirostris	Carpenter, 1978	X	
Atya bisulcata	Carpenter, 1978	X	
A. serrata	Carpenter, 1978	X	
Caridina richtersi	Carpenter, 1978	X	
Axiidae			
Calocaris macandreae	Buchanan, 1963	X	
Campylonotidae			
Campylonotus rathbuni	Yaldwyn, 1966	X	
C. semistriatus	Yaldwyn, 1966	X	
C. capensis	Torti and Boschi, 1973	X	
C. vagans	Torti and Boschi, 1973	X	
Processidae			
Processa edulis	Nöel, 1973	X	
Hippolytidae			
Lysmata seticaudata	Dohrn, 1950	X	
L. nilita	Dohrn and Holthuis, 1950	X	
Chorismus antarcticus	Yaldwyn, 1966	X	
Hippolyte inermis	Veuille *et al.*, 1963	X	
Thor manningi	Bauer, 1986	X	
Pandalidae			
Pandalopsis dispar	Butler, 1964	X	
Pandalus borealis	Butler, 1964	X	
P. danae	Butler, 1964	X	
P. goniurus	Butler, 1964	X	
P. hypsinotus	Butler, 1964	X	
P. jordani	Butler, 1964	X	
P. montagui tridens	Butler, 1964	X	
P. stenolepis	Butler, 1964	X	
P. montagui	Allen, 1963	X	
P. kessleri	Aoto, 1952	X	
Crangonidae			
Argis dentata	Fréchette *et al.*, 1970	X	
Crangon crangon	Boddeke, 1968	X	
C. franciscorum	Gavio (pers. comm.)	X	
I.O. Anomura			
Hippidae			
Emerita analoga	Barnes and Wenner, 1968	X	
E. asiatica	Subramoniam, 1981	X	
S.O. Dendrobranchiata			
Solenoceridae			
Solenocera membranacea	Heergaard, 1967	X	
Subclass Peracarida			
O. Isopoda			
S.O. Anthuridea			
Anthuridae			
Cyanthura carinata	Legrand and Juchault, 1963		X
C. polita	Burbanck and Burbanck, 1974		X
C. profunda	Kensley, 1982		X

Table I. (*Continued*)

Taxa[1]	Reference	Type of sex change: Protandry	Type of sex change: Protogyny
S.O. Flabellifera			
Sphaeromatidae			
Paraleptosphaeroma glynni	Buss and Iverson, 1981		X
Gnorimosphaeroma luteum	Brook (pers. obs.)		X
G. oregonense	Brook *et al.* (this paper)		X
Cymothoidae			
Emetha audouinii	Montalenti, 1941	X	
Anilocra physodes	Montalenti, 1941	X	
A. frontalis	Legrand and Juchault, 1970	X	
Philoscia elongata	Arcangeli, 1925	X	
Nerocila californica	Brusca, 1978	X	
S.O. Epicaridea			
Hemioniscidae			
Hemioniscus balani	Kozloff, 1987	X	
Liriopsidea			
Liriopsis pygmaea	Kozloff, 1987	X	
Cryptoniscidae	Charniaux-Cotton, 1960	X	
Bopyridae			
Munidion pleuroncodis	Markham, 1975	X	
S.O. Oniscoidea			
Oniscidea			
Rhyscotus ortonedae	Jackson, 1928	X	
O. Amphipoda			
S.O. Gammaridea			
Lysianassidae			
Acontiostoma marionis	Lowry and Stoddart, 1986	X	
A. tuberculata	Lowry and Stoddart, 1986	X	
Stomacontion pungapunga	Lowry and Stoddart, 1986	X	
Scolopostoma prionoplax	Lowry and Stoddart, 1986	X	
Ocosingo borlus	Lowry and Stoddart, 1986	X	
O. fenwicki	Lowry and Stoddart, 1986	X	
Stegocephalidae			
Stegocephalus inflatus	Steele, 1967	X	
O. Tanaidacea			
Heterotanais oerstedi	Bückle-Ramirez, 1965		X
Hageria rapax	Modlin and Harris, 1989		X
Leptochelia neapolitana	Ishimaru, 1984		X
L. dubia	Highsmith, 1983		X
L. forresti	Stoner, 1986		X
Class Copepoda			
O. Calanoida			
Calanidae	Fleminger, 1985	X	
Class Cirripedia			
O. Ascothoracica			
Synagoga sandersi	Newman, 1974	X	
Gorgonolaureus muzikae	Grygier, 1981	X	

[1] Taxonomy based on Kozloff (1987). O = order; S.O. = suborder; I.O. = infra-order.

Standing and Beatty, 1977), behavioral (Rees, 1975; Rawlings, 1994), and morphological studies (Hoestlandt, 1973, 1975), there are no records of its reproductive biology or mating system. To examine sex change in *G. oregonense*, we conducted a field census to determine the age and sexual structure of a population and whether social groupings occur. Mature females were raised in the laboratory to observe possible sex changes following hatching of a brood, and juvenile isopods were raised in the presence of adults of each sex to explore the potential for social mediation of sex determination.

Materials and Methods

Field survey

In September 1992, the population structure of *Gnorimosphaeroma oregonense* was examined in a low intertidal channel connecting the mainland to a small piece of land isolated at high tide in Grappler Inlet (48°49′ N, 125°07′ W), Bamfield, British Columbia, Canada. The substrata consisted of a dense covering of clam shells and barnacle- and mussel-covered rocks overlying a fine, silty mud (Rawlings, 1989). A transect was established parallel to the shoreline at a tidal height of 1.6 m above Canadian chart datum, and 10 quadrats (625 cm^2) were placed at 2-m intervals along the transect. All rocks and shells above the mud layer (approximately 7 cm deep) were collected from each quadrat, placed in plastic bags, and within 24 h washed with fresh water and sieved and sorted for isopods.

The isopods were immediately fixed in 70% ethanol and categorized to reproductive stage: juveniles (JUV), with no apparent sexual structures; receptive females, with small, nonfunctional oostegites and with (RFP) or without (RF) penes; mature females, with marsupium of large overlapping oostegites with or without embryos in the marsupium and with (MFP) or without (MF) penes; immature males (IM), with nonfunctional penes and lacking appendix masculina and ciliary tufts on the third and fourth pereopods; and mature males (MM), with functional penes and having appendix masculina and ciliary tufts upon the third and fourth pereopods. Individual body length (from the anterior tip of the cephalon to the posterior tip of the pleotelson) and, when present, penis length (base to the tip of the longest penis) were measured.

Examination of field aggregations. Preliminary field observations indicated that *G. oregonense* was often present in small aggregations arbitrarily defined as a group of more than five individuals within a 36-cm^2 quadrat, under rocks and in barnacle tests. In November 1992, a quadrat was placed over each of 10 aggregations; all the isopods were removed and immediately fixed in 70% ethanol for subsequent measurement and sexing in the laboratory.

Variation in female fecundity with body size. In November 1992, to examine the relationship between fecundity and body size, several randomly selected quadrats were collected (as in the field survey), brought to the laboratory, washed, and sorted for *G. oregonense.* The first 50 mature ovigerous females were fixed in 70% ethanol, measured (body length), and dissected to count brooded embryos.

Laboratory experiments

Culture of receptive and mature females. To monitor changes in external morphology before, during, and after brooding, 20 receptive females in precopula with mature males (guarded) and 20 mature females (not guarded) were collected in July 1992. Each guarded female was checked for the presence of small oostegites (*i.e.*, confirmed to be receptive) and thus represented the female morphology prior to brooding. Mature females were more developmentally advanced, with a functional marsupium containing embryos at various stages of development. Receptive females (with accompanying males) and mature females were cultured in mesh-paneled vials (35-ml vial, 2.5 cm in diameter, with a 4- × 5-cm window of 300-μm Nytex screen). Five vials, containing either receptive females (and accompanying males) or mature females, were randomly allocated to each of eight culture dishes (four replicates per female type) containing 200 ml of filtered (1 μm) seawater and were incubated at 17°C. Within a vial, each isopod was supplied *ad libitum* with the intertidal alga *Ulva fenestrata* for food. Seawater was changed at intervals of 3–4 days, at which time molts were collected and preserved in 5% formalin. When exuviae were observed, the body length of each isopod was measured, and any changes in external morphology were noted.

Sex ratio of developing juveniles. In October 1992, two simultaneous experiments were conducted to investigate the effect of the presence of mature males and mature females on the sexual development of juvenile *G. oregonense.* The effect of adult males was examined by adding either 0, 1, or 5 mature male isopods to a culture dish (500-ml dish containing 250 ml seawater) with 10 developing juveniles (five replicate dishes/treatment) at 17°C. To ensure that juvenile isopods were reproductively immature, only individuals with a body length of 3–4 mm and no external evidence of reproductive structures were used. The isopods were provided *ad libitum Ulva fenestrata* and clean clam shells for substratum. Seawater was changed every 3–4 days, at which time mortality was noted and, if necessary, mature males were replaced. To ensure that all surviving juveniles had the same length of exposure time to adults, dead juveniles were not replaced.

To examine the effect of mature females on the reproductive development of juveniles, the same procedures were followed, but the density of female rather than male *G. oregonense* was manipulated.

Scanning electron microscopy

To observe and compare reproductive structures of male and female *G. oregonense,* individuals were fixed for scanning electron microscopy. Specimens were anesthetized (in three parts seawater to one part carbonated water) and refrigerated at 4°C for 1 h. When relaxed, they were fixed in 2.5% glutaraldehyde for 2 h, washed for 30 min in distilled water, and postfixed in 1% osmium tetroxide for 1 h. After fixation, the specimens were dehydrated for 30 min at each dilution in

an ethanol series (30%, 50%, 70%, 95%, and 100% ethanol in distilled water), critical-point dried in carbon dioxide (31°C, 1100 psi), sputter coated with gold for 10 min, and photographed using a Jeol JSM-35 scanning electron microscope.

Results

Field survey

Examination of field aggregations. Gnorimosphaeroma oregonense was patchily distributed among the shell debris and barnacle- and mussel-covered rocks in Grappler Inlet. In September 1992, densities varied from 80 to 13,136 individuals per square meter. Because two of the ten samples were extremely large (n = 642 and 821), eight samples were fully analyzed for population structure and the two large samples were subsampled by randomly mixing the isopods in 1 l of water and removing a fraction of the volume from the agitated solution (Wrona *et al.*, 1982). Of the 329 individuals examined, 78% were juveniles, 15% females (mature and receptive), and 7% males (Fig. 1). The operational sex ratio (mature males to receptive females) was 1:1.

Marked sexual dimorphism was evident among male and female *G. oregonense* (Fig. 1; Table II). Mean body length differed significantly among females and males (ANOVA; F = 307.94, $P < 0.0001$). Receptive females (RFP and RF) were significantly smaller than mature females, and mature females with penes (MFP) were not different in body length from mature females without penes (MF) (Table II). There was very little overlap in size range between mature females and mature males (Fig. 1).

Penes were present on all males, on 30% of mature females (n = 80), and on 11% of receptive females (n = 38) sampled in September 1992. All mature females (n = 40) collected in November 1992 had small penes. No other differences in body morphology were evident.

Penis length was related to sex and reproductive maturity. Mature females (n = 35) had penes that were significantly smaller (0.07 ± 0.005 mm) than penes of immature males (n = 16; 0.12 ± 0.015 mm) and mature males (n = 48; 0.45 ± 0.009 mm) (Kruskal-Wallis: H = 75.17, $P < 0.001$) (Figs. 2 and 3). Penis morphology also differed among reproductive stages. The penes of mature males were not only longer, but also had a pore or sphincter on the distal tip (Fig. 4) that was not present on penes of immature males or receptive and mature females.

Field aggregations of *G. oregonense* varied in number from 6 to 36 individuals and consisted primarily of juveniles and immature males (Fig. 5A). The presence of receptive and mature females was strongly correlated ($P < 0.001$) with the presence of mature males (Fig. 5B; $Y = 3.088X + 0.265$, $r^2 = 0.757$, n = 10, where Y is the number of mature males, and X is the number of receptive and mature females), suggesting that males and females were aggregating socially.

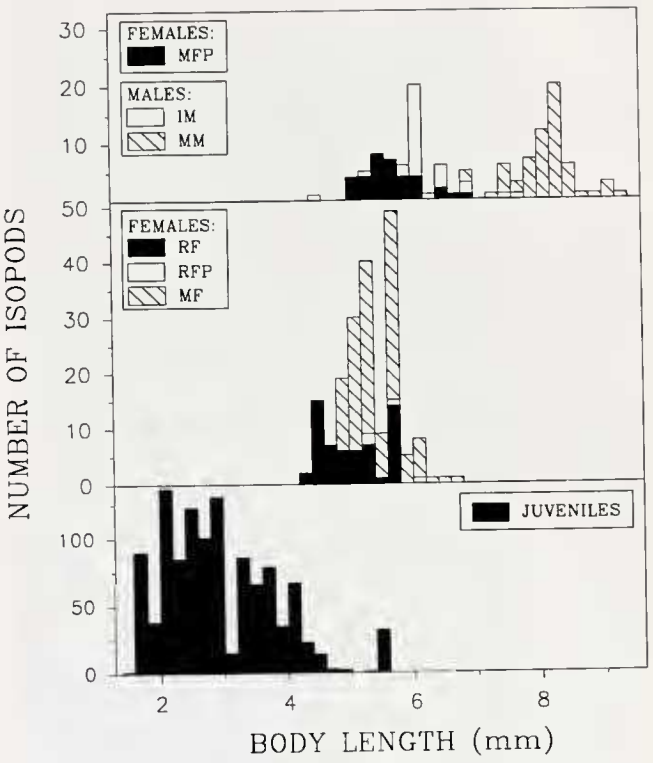

Figure 1. Size-frequency histogram of the body lengths of *Gnorimosphaeroma oregonense* collected from Grappler Inlet, September 1992. The population is classified into seven reproductive categories based on external morphology: juveniles (JUV)—no obvious sexual features; receptive females—small oostegites, no penes (RF) or with penes (RFP); mature females (MF)—large overlapping oostegites, no penes; mature females (MFP)—with penes; immature males (IM)—with penes, no oostegites; and mature males (MM)—penes, ciliary tufts on the third and fourth pereopods, and paired appendix masculina.

Variation in female fecundity with body size. Brood size in mature females varied from 13 to 42 and was associated with differences in female body size. Embryo number was positively correlated with female body length (Fig. 6; $Y = 17.69X - 66.90$, $r^2 = 0.67$, n = 49; where Y is the number of embryos, and X is the body length in millimeters).

Laboratory experiments (see below) showed no evidence of repeat brooding, indicating that *G. oregonense* broods only one clutch of embryos. Field collections provide corroborative evidence for a single brood, because there was no evidence of a bimodal frequency distribution of receptive or mature females suggestive of repeat brooding (Fig. 1).

Laboratory experiments

Culture of receptive females. Of the 20 females, 19 were securely guarded by mature males and had small ooste-

Table II

Differences in mean (±standard deviation) body length (mm) among reproductive stages of Gnorimosphaeroma oregonense *collected from Grapper Inlet, September 1992*

Sex/Stage	Sample size (n)	Body length[1]
Receptive females	37	4.98 ± 0.38^{a}
Mature females (no penes)	51	5.61 ± 0.42^{b}
Mature females (penes)	31	5.72 ± 0.44^{b}
Immature males	19	6.21 ± 0.72^{c}
Mature males	50	8.14 ± 0.45^{d}

[1] Body lengths of reproductive stages with the same superscript are not significantly different ($P > 0.05$) from one another (ANOVA, *a posteriori* Tukey test).

ANOVA: between sex/stage df MS Error MS F-ratio P
4 65.998 0.214 307.943 <0.0001

gites; the remaining female was mature and was removed from the experiment. Because only isopods with small oostegites occur in precopula, or are securely grasped by mature males, they were defined as receptive. Receptive females continued to be guarded by their males, female dorsum against the male venter, until the biphasic female reproductive molt was complete 1 to 13 days after collection. A biphasic molt was observed in all molts for juveniles, females, and males. The reproductive molt consisted of two phases, with the posterior portion of the female molting first, followed, within 24 h, by the anterior portion. Copulation was observed on one occasion following the first phase of the biphasic molt and was presumed to occur at this stage in all other pairs. Additionally, in all cases, mature males abandoned their females following the second half of the biphasic molt, suggesting that copulation had occurred.

The molt from the receptive to the mature female form was accompanied by an increase in body length and the formation of a functional marsupium (Fig. 7A, B). Most males (n = 16, 80%) died shortly (10–30 days) after copulation, and some female mortality occurred during brooding (n = 6, 31%) and later development (n = 4, 21%). Embryos became visible in the marsupium 1 to 3 days after completion of the anterior molt and developed at 17°C to hatchlings (mancas) in 8 to 10 weeks. No females molted during this brooding period (Fig. 7B). After the hatching of juveniles from the marsupium (days 100–120) (Fig. 7), all females molted to the immature male form, with the loss of oostegites and further development of penes. Females without penes already present (n = 2, 10%) developed them at this molt. Over the next 60 days (days 120–180), 90% of surviving females (n = 10) underwent a second molt, increasing in size but remaining in the immature male form. After 225 days, eight (42%) individuals molted to the mature male form with long penes, ciliary tufts on the third and fourth pereopods, and paired appendix masculina.

Culture of mature females. Brooding females demonstrated a pattern of morphological change similar to that of receptive females, although molting was less synchronous (Fig. 8A, B). During the first 90 days in the laboratory, all surviving brooding females (n = 17, 85%) released their broods, molted, and underwent a concomitant increase in size (Fig. 8B). These females usually molted within 3 to 4 weeks after juveniles hatched from the marsupium. Of these 17 females, 16 molted into immature males and 1 molted directly into a mature male. By day 70, two of the immature males also molted to the mature male form.

During the next 100 days in the laboratory, all surviving immature males molted a second time, but retained their immature male form (Fig. 8A, B). After 160 days, four of the surviving immature males (n = 13) molted to the mature male form (Fig. 8A, B), and two individuals (15% of survivors) molted for a third time as immature males, but died shortly thereafter. Consequently, after 200 days in the laboratory, seven of the surviving 14 brooding female isopods showed direct evidence of sex change.

There was considerable variation among females in the number of molts taken to become a mature male (Fig. 9). One mature female molted directly into a mature male, others took two or three molts (Fig. 8), and some remained

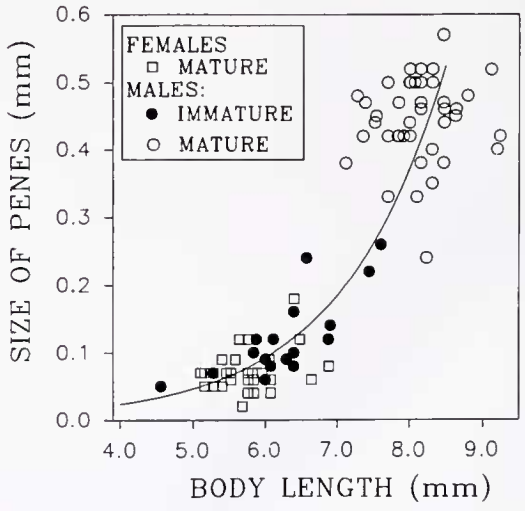

Figure 2. Relationship between penis length and body length for mature receptive and mature female (n = 33), immature male (n = 17), and mature male (n = 47) *Gnorimosphaeroma oregonense* collected from Grappler Inlet, September 1992. Penis length varied with body size according to the relationship $Y = 0.0014 \times 10^{0.301X}$, $r^2 = 0.82$.

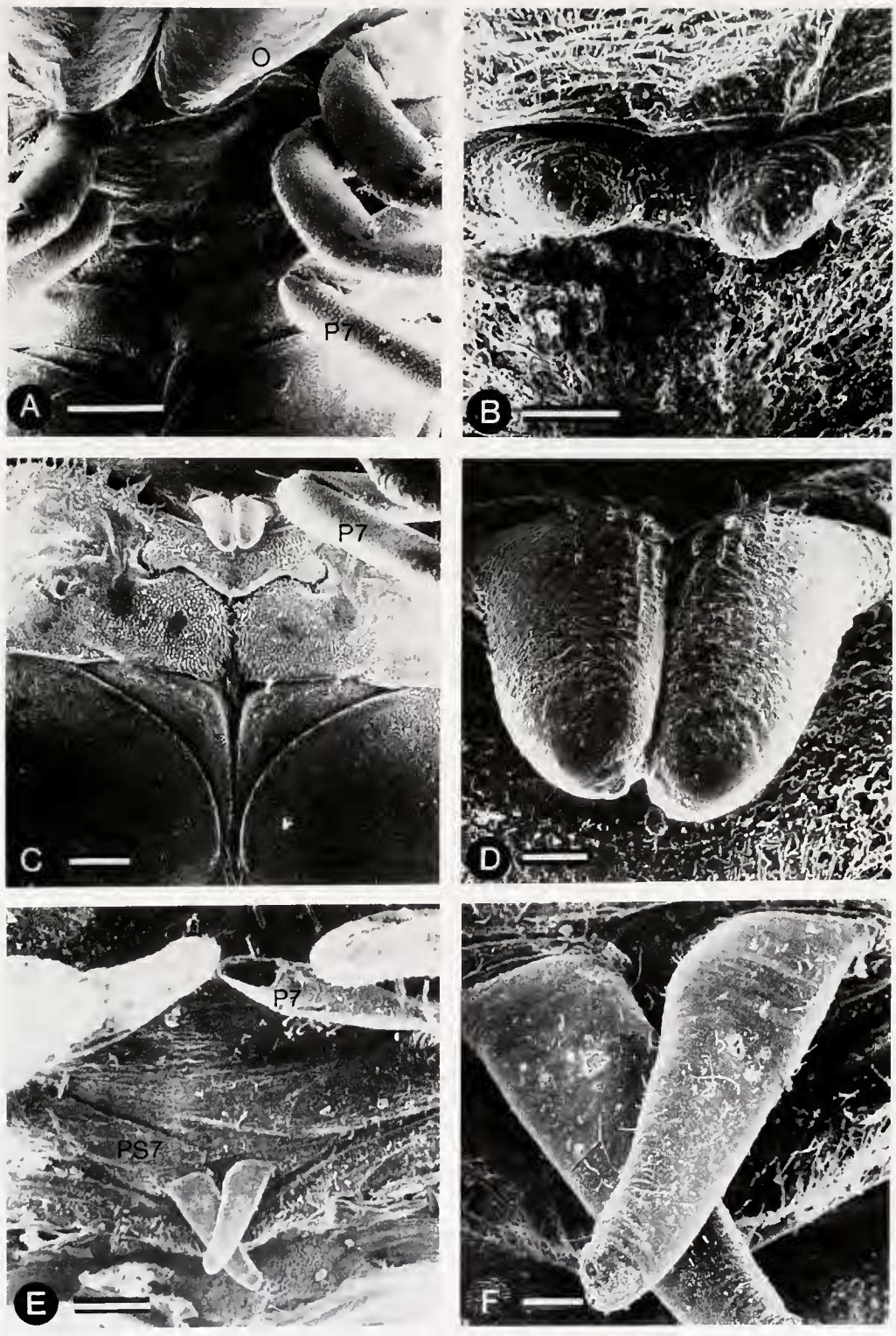

Figure 3. Scanning electron micrographs illustrating the development of penes on mature female (A, B), immature male (C, D), and mature male (E, F) *Gnorimosphaeroma oregonense*. For each reproductive stage, a low-magnification (A, C, E; scale bar = 0.30 mm) and high-magnification view (B, D, F; scale bar = 0.06 mm) of the paired penes are shown. Large oostegites and developing penes are shown in the mature female (A). O, oostegite arising from the 4th pereopod; P7, 7th percopod; PS7, 7th pereonal segment.

Figure 4. Scanning electron micrograph of the paired penes, with openings of the duct at the distal ends, of a mature male *Gnorimosphaeroma oregonense* (scale bar = 0.06 mm).

in the immature form after three molts. In the September 1992 field census, a few smaller males were present, similar in size to the one-molt mature males, suggesting that molt number varies in the field as well as in the laboratory. This variability was not associated with female size at brooding or with the precocial development of penes.

Sex ratio of developing juveniles. The sex ratio of developing juveniles was unaffected by the presence of mature male and mature female *G. oregonense* (Fig. 10). Due to the high mortality (50–51%) of juveniles over the course of the 52-day culture period in both experiments, replicates within a treatment were pooled for statistical analysis. The presence of adult males ($X_{0.05,4}^2 = 1.207$, $P > 0.80$) or brooding females ($X_{0.05,4}^2 = 1.590$, $P > 0.80$) had no significant effect on the sexual development of juveniles.

The results of these experiments showed that not all *G. oregonense* are protogynous. Although a large percentage of individuals could not be sexed at the end of the culture period, 63.3 ± 3.7% ($n = 126$) of juveniles developed into immature males, with penes and no oostegites. In contrast, only 8.2 ± 3.4% ($n = 16$) developed into receptive females. The remaining juveniles ($n = 57$) had not matured to a stage exhibiting external reproductive structures before the experiment was terminated. Immature males that were isolated and maintained in plastic vials after the end of the experiment continued to develop to the mature male form, and none became females.

Discussion

The results of this study provide direct evidence of protogynous sex change within the isopod *Gnorimosphaeroma oregonense*. Field surveys showed sexual size di-

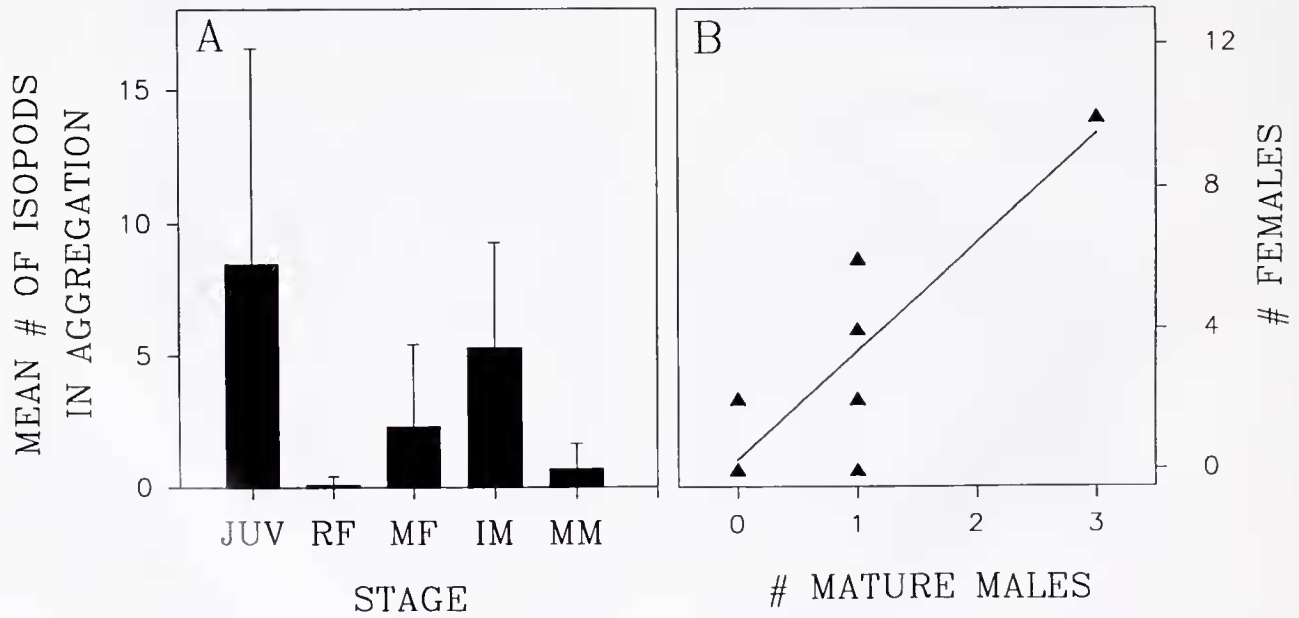

Figure 5. Number of individuals present in aggregations of *Gnorimosphaeroma oregonense* sampled from beneath rocks in Grappler Inlet, November 1992. (A) Mean number of isopods (+ standard deviation) of each stage in aggregations ($n = 10$); (B) relationship between the number of receptive and mature females (RF, RFP, MF, and MFP inclusive) and the number of mature males (MM) in aggregations ($Y = 3.088X + 0.265$; $r^2 = 0.75$, $n = 10$).

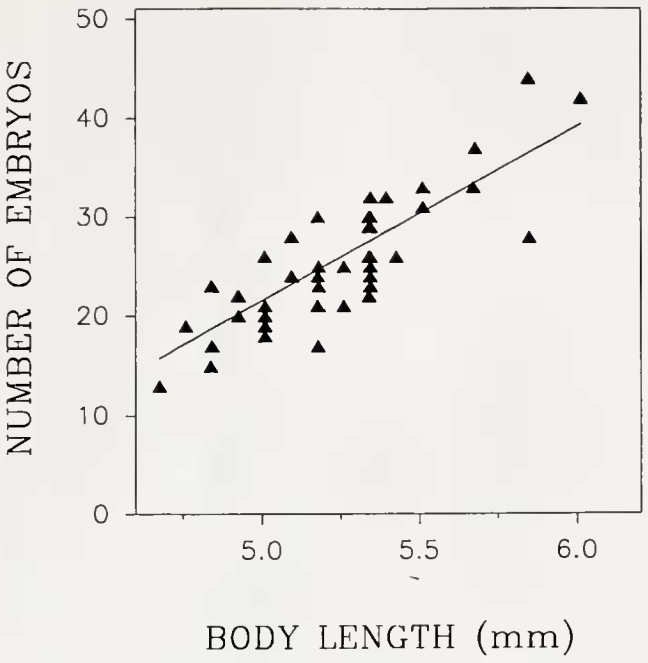

Figure 6. Fecundity of *Gnorimosphaeroma oregonense* collected from Grappler Inlet, November 1992 ($Y = 17.69X - 66.90$, $r^2 = 0.67$, $n = 49$). Females carrying embryos at all stages of development are included.

morphism, with males larger than females, and the presence of male and female characteristics within individual *G. oregonense*. Laboratory experiments showed that, following brood release, females either molt directly into mature males or pass through an immature male phase to become mature males, corroborating that *G. oregonense* is protogynous.

Selection favoring protogyny

Although a number of other crustaceans are reported to be protogynous (Table I), little is known about the reproductive biology and life-history characteristics associated with protogyny in this group. The isopod *Cyanthura carinata* and the tanaid *Hargeria rapax* are the only protogynous species in which the reproductive cycle and population dynamics have been thoroughly examined (Bamber, 1985; Modlin and Harris, 1989; Kneib, 1992). Within the tanaidaceans, protogyny appears to be associated with three life-history features: (1) low mobility of females, (2) low abundance of males due to high mortality, and (3) intense competition among males for access to females (Highsmith, 1983). Ghiselin (1969) proposed that sequential hermaphroditism will be favored by selection when the relative reproductive success of a male or female differs with size or age. Based on Ghiselin's size advantage model (1969), protogyny should be favored in species in which male reproductive success is dependent on large body size, increased experience, or female mate choice (Warner, 1975; Warner, 1988).

Precopulatory mate guarding in *G. oregonense* may be a primary selective force favoring protogyny. In many isopod species, males actively guard females in a precopulatory embrace (Wilson, 1991). Sexual dimorphism and precopula have been associated with protogyny in *G. oregonense*, *G. luteum* (Brook, personal observation), *Cyanthura carinata* (Bamber, 1985), and *Paraleptosphaeroma glynni* (Buss and Iverson, 1981). Active precopula has also been reported in gonochoristic species such as *Asellus aquaticus* (Manning, 1975, 1980), *A. meridianus* (Steel, 1961), *Jaera italica* and *J. nordmanni* (Veuille, 1980). Larger males may guard females or a resource more effectively or be able to remove a female from a smaller competitor, giving a higher reproductive success to larger males than to smaller ones (Ridley and Thompson, 1979; Ridley, 1983; Warner, 1988). Although the *G. oregonense* population sampled had more mature males than receptive females (Table II), all receptive fe-

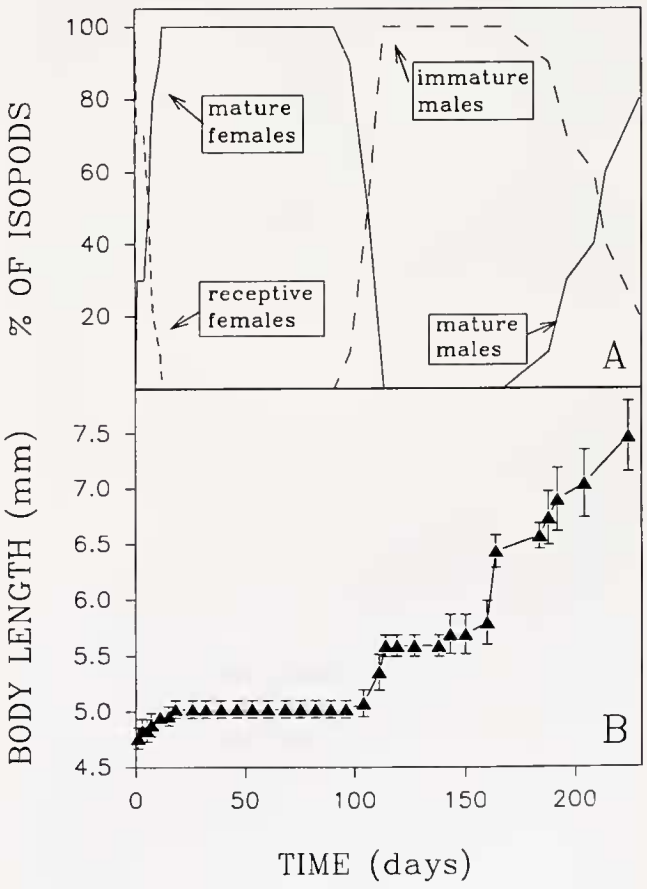

Figure 7. Changes in the external morphology of receptive female *Gnorimosphaeroma oregonense* that survived 225 days in the laboratory or that molted to the terminal mature male stage. (A) Percentage of isopods ($n = 10$) at each reproductive stage; (B) mean body length (±SE) of these individuals, for each observation interval.

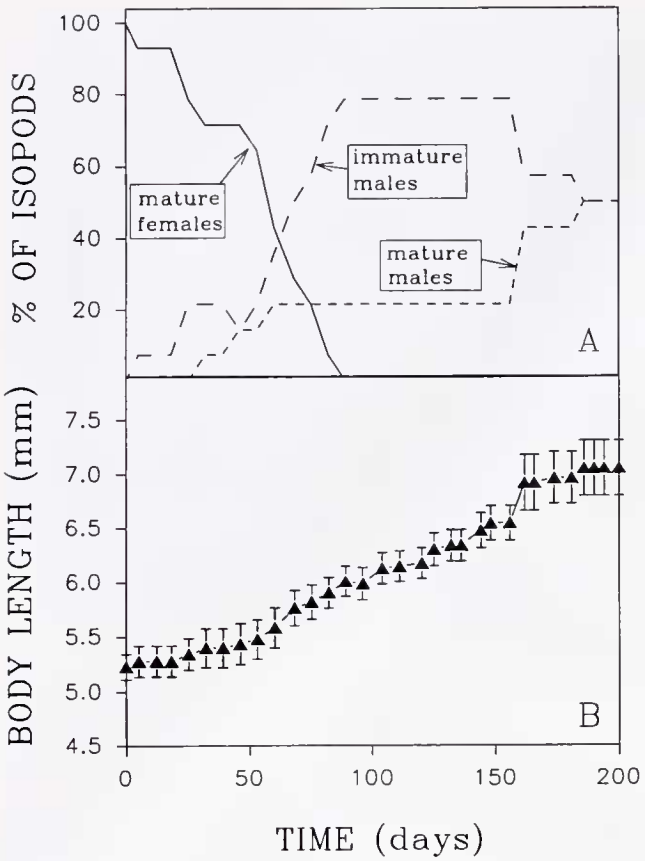

Figure 8. Changes in the external morphology of mature female *Gnorimosphaeroma oregonense* that survived 200 days in the laboratory or that molted to the terminal mature male stage. (A) Percentage of isopods (n = 14) at each reproductive stage; (B) mean body length (±SE) of these individuals, for each observation interval.

males are not necessarily nearing the reproductive molt. The operational sex ratio may thus be skewed towards males, further increasing male-male competition for females that are nearing the reproductive molt, as well as selection for larger male size.

Active precopulatory guarding has not been observed in protandrous isopods. Precopulatory behavior may involve the passive attachment of the dwarf male onto the larger female, as seen in the parasitic epicarids (Wilson, 1991). There is no evidence, however, that this excludes other males from mating with females in these species. In the protandrous hippolytid shrimp *Thor manningi* (Bauer, 1986), available evidence suggests that male reproductive success may be independent of size. Although larger males are more frequently paired with females in the partially protandrous shrimp *Athanas kominatoensis* (Nakashima, 1987), some small males do successfully mate with females. Female fecundity increases with body size, and thus a small male will increase its reproductive output. In both protandrous and protogynous crustaceans, female fecundity is usually positively correlated with body size (Charnov, 1979; Bauer, 1986; Nakashima, 1987; Bamber, 1985; Buss and Iverson, 1981; Kneib, 1992; this study). It appears, therefore, that the advantage gained by larger males in the protogynous crustacean mating system overrides the selective force of increased female fecundity with increased female body size.

Socially and environmentally mediated protogynous sex change

Protogynous sex change has been suggested to be socially mediated in the tanaids *Leptochelia dubia, Hargeria rapax,* and *Heterotanais oerstedi* and in the isopod *Paraleptosphaeroma glynni* (Highsmith, 1983; Modlin and Harris, 1989; Bückle-Ramirez, 1965, as cited in Gardiner, 1975; Buss and Iverson, 1981). In all these studies, protogynous sex change occurred (though not exclusively) when adult males were absent (Modlin and Harris, 1989). In our study, the presence of adult male and female *G. oregonense* had no significant effect on the sexual development of juveniles. Also, laboratory-cultured females changed sex to either the immature or mature male form, regardless of whether mature males were present. The absence of socially mediated sex change is less surprising if, as suspected, female *G. or-*

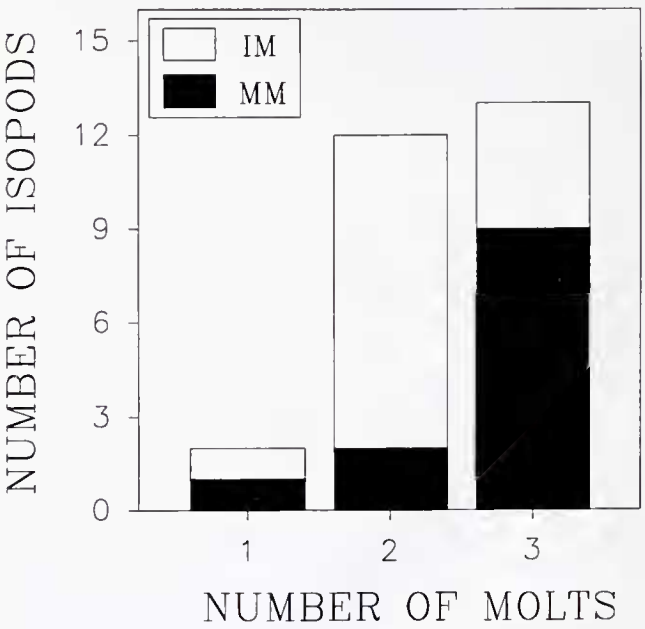

Figure 9. Number of molts required by mature female *Gnorimosphaeroma oregonense* to develop into mature males over 210 days in the laboratory. Solid bars refer to isopods that molted to the mature male (MM) condition over this time period. Hollow bars refer to individuals that underwent a variable number of molts as immature males (IM), but had not molted to the mature male form by the end of the experiment.

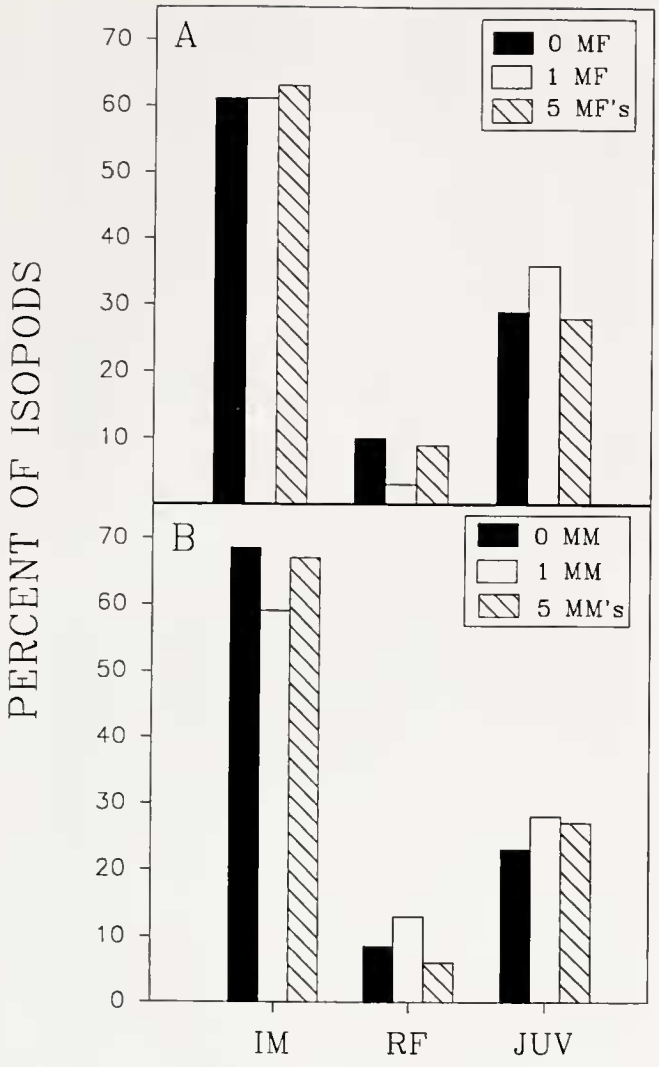

Figure 10. Percentage of juvenile isopods that developed into males or females or remained as juvenile *Gnorimosphaeroma oregonense*, after exposure to three different densities of (A) mature females (MF) (0, 1, or 5 females) or (B) mature males (MM) (0, 1, or 5 males) for 52 days. IM = immature males, RF = receptive females, JUV = juveniles.

egonense brood only one clutch of embryos. In that case, reproductive success could be increased only if a female changed sex to a male and contributed to the gene pool as both female and male. Because males may be able to inseminate multiple females in the time required for a female to brood a single clutch, sequential hermaphroditism is clearly potentially advantageous.

Unlike other protogynous isopod species, *G. oregonense* exhibited plasticity in the number of molts required for a female to undergo sex change. Buss and Iverson (1981) reported that the isopod *Paraleptosphaeroma glynni* could molt from a mature female to a mature male in one molt. The plasticity in *G. oregonense* may be adaptive—allowing an individual to optimize the timing of maturity, perhaps in response to the presence or absence of other adult males. Additionally, mature males are in their terminal molt and have a short life expectancy; therefore, plasticity of development may provide a means to optimize reproductive success. In the protandrous shrimp *Pandalus jordani*, individuals alter the age at which they change sex in response to year-to-year variations in population structure (Charnov, 1978).

Not all *G. oregonense* juveniles are protogynous: only a small percentage of juveniles developed into females in the laboratory. As in other protogynous crustaceans, some individuals first develop to breeding females and later change to reproducing males (termed secondary males); whereas others (termed primary males) remain as males, lacking any female characters, throughout their lives (Highsmith, 1983; Bamber, 1985; Stoner, 1986; this study). It appears that *Paraleptosphaeroma glynni* may be completely protogynous, because no small males were found in the field population (Buss and Iverson, 1981). In some species of protandrous caridean shrimp, all individuals undergo sex change. Other species show varying degrees of sex change, with some individuals changing sex and others remaining strictly female (primary females) throughout their lives (Bauer, 1986). The selective advantages associated with the difference between primary and secondary males or females have not been determined.

Although the sex of juveniles could possibly be determined very early in development, it is more likely to be controlled by environmental factors such as temperature or photoperiod or perhaps by the presence of immature males rather than adult females or males. Seasonal patterns in the occurrence of protogyny have been documented in the isopod *Cyanthura carinata* (Legrand and Juchault, 1963) and in the tanaid *Heterotanais oerstedi* (Bückle-Ramirez, 1965, as cited in Gardiner, 1975). The influence of environmental (epigenetic) factors such as temperature, photoperiod, and parasitism on the sex determination of Crustacea has been observed in isopods, amphipods, and copepods (Ginsberger-Vogel and Charniaux-Cotton, 1982; Juchault and Mocquard, 1989). Sex change has also been experimentally induced by androgenic gland grafting and removal in some malacostracans that are not hermaphroditic in nature (Charniaux-Cotton, 1975).

Although life-history theory predicts that protandry or protogyny should occur in many crustaceans (*e.g.*, many amphipods are sexually dimorphic and exhibit precopulatory guarding behavior; Ridley, 1983), protogynous sex change has not been reported. Perhaps the incurred costs of sex change in these and other gonochoristic crustacean species outweigh the benefits associated with increased reproductive output.

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